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Genetic Localisation of MRX27 to Xq24-26 Defines Another Discrete Gene for Non-Specific X-Linked Mental Retardation

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A large family with non-specific X-linked mental retardation (MRX) was first described in 1991 [Glass et al., 1991], with a suggestion of linkage to Xq26-27. The maximum lod score was 1.60 ($\theta = 0.10$) with the *F9* locus. The localisation of this MRX gene has now been established by linkage to microsatellite markers. Peak pairwise lod scores of 4.02 and 4.01 ($\theta = 0.00$) were attained at the *DXS1114* and *DXS994* loci respectively. This MRX gene is now designated *MRX27* and is localised to Xq24-26 by recombination events detected by *DXS424* and *DXS102*. This regional localisation spans 26.2 cM on the genetic background map and defines another distinct MRX interval by linkage to a specific region of the X chromosome.

KEY WORDS: MRX27, X-linked mental retardation, linkage

INTRODUCTION

X-linked mental retardation (XLMR) is a major contributor to the excess of retarded males in the population [Lehrke, 1972]. The fragile X syndrome (fraX) alone accounts for just less than half of all X-linked mental retardations [Opitz, 1986]. Other clinically defined syndromal forms of XLMR, identifiable on the basis of a recognizable pattern of physical anomalies or XLMRs with neuromuscular or metabolic involvement [Neri et al., 1994] are less frequent. Non-specific X-linked mental retardation (MRX) together with fraX may account for up to 25% of all mental retardation in man [Opitz, 1986]. MRX is defined as intellectual handicap segregating in an X-linked manner but without consistent somatic or clinical manifestations [Kerr et al., 1991]. Determination of non-overlapping gene localisations is the only means of classifying these non-syndromal forms of mental retardation and establishing the minimum number and distribution of MRX loci along the X chromosome.

Genes localised in large families segregating MRX are assigned an MRX number once linkage is supported by a two-point or multipoint lod score of +2 or greater [Mulley et al., 1992]. There are now many reports of MRX gene localizations in large pedigrees demonstrating that clinically homogeneous MRX is genetically heterogeneous [Gedeon et al., 1996a]. The familial non-specific XLMR associated with expansion of (CCG)n repeats at FRAXE represents one MRX [Knight et al., 1993] and the associated gene is now known [Gecz et al., 1996]. Four non-overlapping regions for MRX have already been established by linkage [Gedeon et al., 1994a]. The *MRX24* locus has been recently localised by linkage and represents an additional MRX locus identified by linkage mapping [Martinez et al., 1995].

The following study was undertaken to establish the gene localisation in a previously described family segregating MRX. Earlier attempts to map the MRX gene in this family using RFLP markers spanning the X chromosome, yielded a hint of linkage to the *F9* locus at Xq26-27 with a peak lod score of 1.6 ($\theta = 0.10$) [Glass et al., 1991]. The gene in this family has now been designated *MRX27*, having attained a significant lod score greater than +2, and delineates by linkage another discrete gene for non-specific mental retardation on the X chromosome.

MATERIALS AND METHODS

The Family (GLA 2617)

Clinical findings in the GLA 2617 family (Fig. 1) with MRX have been described earlier [Glass et al., 1991]. Delayed language acquisition and behavioural difficulties were the only manifestations associated with the

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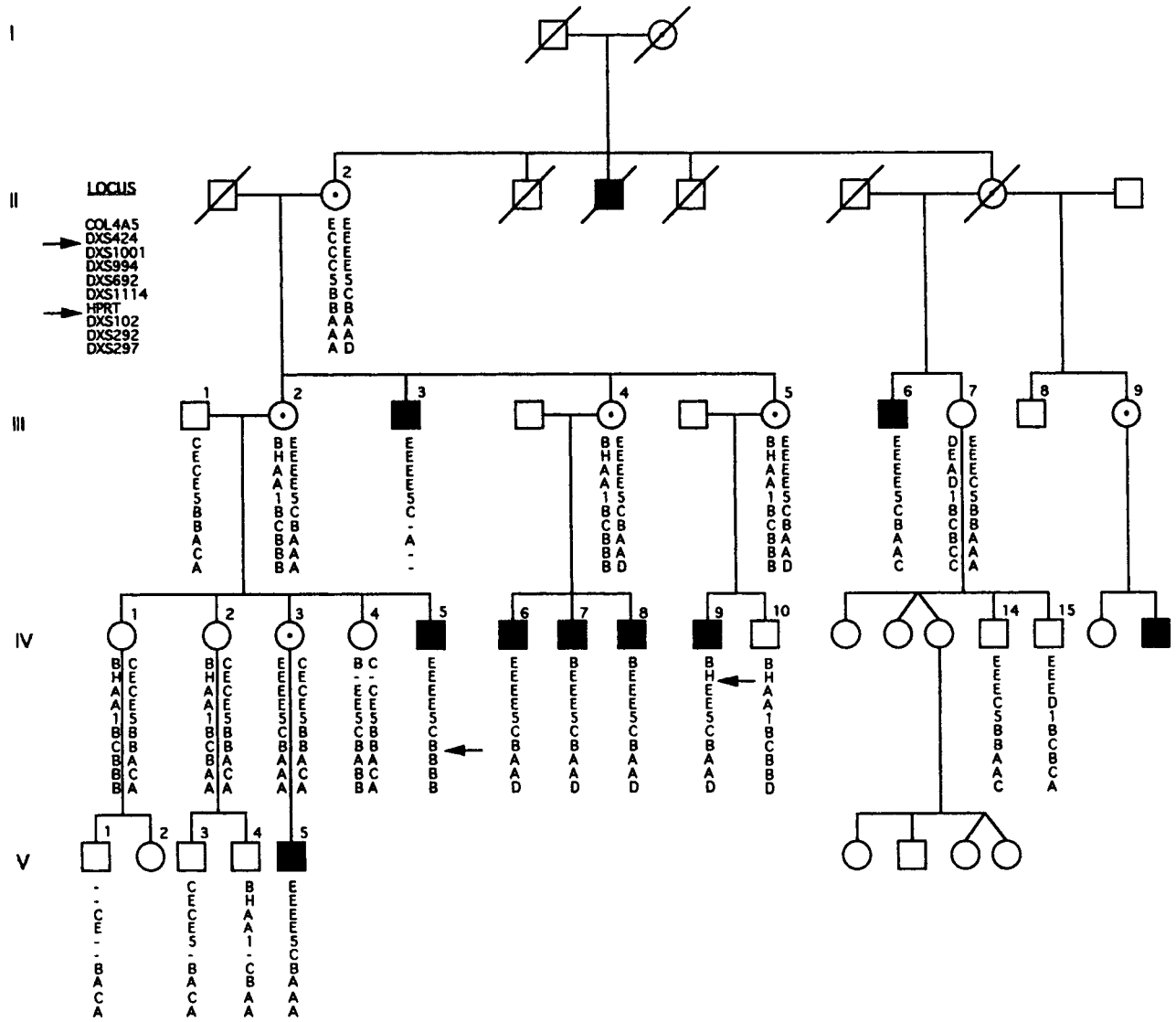

Fig. 1. Pedigree of the family described by Glass et al. [1991] and now designated MRX27. ■ = affected male; ⊙ = obligate carrier female; □ = unaffected male; ○ = unaffected female.

mild to moderate mental retardation in affected males. Two of the carrier females (III-4 and IV-3) had borderline intellectual handicap and required special schooling. Segregation of a folate sensitive fragile site in distal Xq was excluded by chromosome analysis [Glass et al., 1991].

DNA and Linkage Analysis

DNA samples from 24 members of the family (Fig. 1) were retested to establish linkage and an accurate regional gene localisation using microsatellite markers now available from Xq22-Xq27. Samples were genotyped for microsatellite markers as described elsewhere [Gedeon et al., 1994b].

Linkage analyses were carried out using the MLINK program of the LINKAGE package. The frequencies of marker alleles segregating in the family were assumed to be equal for microsatellite loci. The disease gene frequency was set at 10^{-4} and the analysis was carried out under the assumption of X-linked recessive inheritance. The location of *MRX27* was determined against a refined genetic background map prepared from the CEPH database [Gedeon et al., 1996b]. The order of and distance (in cM) between relevant loci as used in this investigation was: *COL4A5*-20.0-*DXS424*, *DXS1001*-14.6-*DXS994*, *DXS692*-4.3-*DXS1114*, *HPRT*-7.3-*DXS102*-1.7-*F9*-18.1-*DXS292*-4.4-*DXS297*.

RESULTS

Two point lod scores were generated between the gene segregating in the GLA2617 family and ten marker loci (Table I) spanning Xq22.3-q27.3. Evidence for linkage of *MRX27* to the region adjacent to the *F9* locus was confirmed by peak pairwise lod scores of

TABLE I. Pairwise Lod Scores Between MRX27 and Marker Loci Spanning Xq22.3-q27.3

	θ								
Loci	0.001	0.01	0.05	0.1	0.2	0.3	0.4	Z max	θ
COL4A5	−6.22	−3.26	−1.30	−0.57	−0.01	0.16	0.15	0.17	0.35
DXS424	0.20	1.16	1.66	1.70	1.42	0.95	0.43	1.71	0.09
DXS1001	1.07	2.02	2.46	2.42	1.99	1.35	0.65	2.47	0.07
DXS994	4.00	3.94	3.66	3.31	2.55	1.71	0.81	4.01	0.00
DXS692	2.42	2.39	2.23	2.03	1.61	1.14	0.52	2.43	0.00
DXS1114	4.01	3.95	3.66	3.28	2.47	1.58	0.62	4.02	0.00
HPRT	2.44	2.40	2.26	2.06	1.64	1.18	0.64	2.44	0.00
DXS102	−0.56	0.41	0.98	1.11	1.04	0.81	0.46	1.12	0.13
DXS292	−2.96	−0.99	0.26	0.66	0.84	0.72	0.44	0.84	0.20
DXS297	−8.63	−4.65	−1.96	−0.89	−0.02	0.27	0.25	0.29	0.35

4.02 and 4.01 ($\theta = 0.00$) at the *DXS1114* and *DXS994* loci, respectively. The *DXS1122, DXS300, DXS294,* and *DXS984* loci were uninformative in this region.

The *MRX27* gene is localised to Xq24-26 by flanking recombination events detected in the affected males IV-9 at *DXS424* and IV-5 at *DXS102*. These loci represent the proximal and distal boundaries for the *MRX27* gene localisation. The estimated genetic distance between the flanking markers *DXS424* and *DXS102* is 26.2 cM.

DISCUSSION

Linkage analysis with 14 RFLP loci initially suggested localisation of the MRX gene segregating in this family close to the *F9* gene locus with a lod score of 1.6 ($\theta = 0.10$) [Glass et al., 1991]. Peak lod scores greater than +2 now provide significant evidence for linkage at a number of loci in the Xq24-q26 region. The MRX gene causing mental retardation in this family has been designated *MRX27*. Recombinant events in affected males define the proximal and distal limits to the *MRX27* gene localisation between *DXS424* and *DXS102*. An RFLP marker, *DXS51,* within this interval was previously reported with high negative lod scores which excluded close linkage to this marker [Glass et al., 1991]. The confusion of some samples, uncovered by highly informative microsatellite markers and confirmed by re-extracting DNA from stored lymphoblastoid cells, accounts for this discrepancy.

Gene mapping has led to the regional localisation of MRX genes in more than 30 families. The clustering of regional localisations at the centromere has been noted before [Kerr et al., 1992]; however, at least 8 discrete MRX genes are now known to be spread along the X chromosome [Gedeon et al., 1996a]. These are defined by, in order from pter to qter, MRX24 [Martinez et al., 1995], MRX2 [Hu et al., 1994], MRX10 [Gedeon et al., 1996a], MRX1 [Gedeon et al., 1996a], MRX30 [Donnelly et al., 1996], MRX27 [this study], FRAXE [Knight et al., 1993], and MRX3 [Gedeon et al., 1991]. Genetic localisation of the *MRX27* gene to a discrete interval defines a distinct MRX localisation on the X chromosome. This localisation does not currently overlap with any other published MRX gene except perhaps for the ill-defined localisation of *MRX6*. No boundaries to *MRX6* have been formally described [Kondo et al., 1991].

The gene for a severe mental retardation syndrome [Gustavson et al., 1993] has been mapped to an interval flanked by the markers *DXS424* and *DXS297* [Malmgren et al., 1993]. The gene for another XLMR syndrome [Pettigrew et al., 1991] is localised between *DXS425* and *F9* [Huang et al., 1992]. The gene for Börjeson-Forssman-Lehmann syndrome (BFLS) is linked to this region also, and has been described with mild to moderate mental retardation. *BFLS* maps between *DXS425* and *DXS105* [Gedeon et al., 1996b]. The localisations for these syndromal XLMRs overlap with MRX27. The possibility that these disorders are allelic or manifestations of a contiguous gene syndrome cannot be excluded, although more than one gene for XLMR may map to this region of the X chromosome.

Non-specific X-linked mental retardations have been shown to be a genetically heterogeneous group of conditions that cannot be clinically subdivided. The mapping of MRX27 has led to the delineation of a new interval for an MRX gene which highlights the value of studies of new MRX families and the refinement of localisations for existing families. Until a more direct technique for determination of the specific MRX gene segregating within a family is discovered, all MRX families must be considered as separate entities for linkage studies. The discovery of (CCG)n repeat amplification in families with non-specific FRAXE mental retardation has permitted direct detection of the mutation in these pedigrees [Knight et al., 1993 where the defect is within the *FMR2* gene; Gecz et al. 1996].

The positional candidate gene approach could theoretically lead to the identification of MRX genes. Candidate genes are those which lie within the interval delineated by gene mapping and which are expressed in neural tissue. One source of candidate genes are the expressed sequence tags (ESTs) derived from human brain cDNA libraries and which are being physically mapped [Parrish and Nelson, 1993; Mazzarella and Srivastava, 1994]. Direct mutation analysis of patient material with candidate genes will be essential for the identification of MRX genes. Regional localisations established by linkage and delineated by flanking markers are often very broad, however, and unless a related family of genes can be implicated in MRX the search for the gene in each family may encompass an overwhelming number of potential candidate genes to be screened by existing technology.

The proposal that a lymphoblastoid cell line repository of MRX patients be established [Mandel, 1994] would enable rapid, simultaneous screening of a gene of known position through probands from several families exhibiting overlapping gene localisations. In this manner samples from families too small to achieve a lod score of +2 or greater, and families with syndromal forms of X-linked mental retardation, can be included into mutation screening protocols. Eventually it is envisaged that overlapping MRX regional localisations will be lumped or split as genes for MRX are identified. This will lead to the determination of the number of X-linked genes for mental retardation and show their distribution along the X chromosome.

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